THE KANSAS UNIVERSITY SCIENCE BULLETIN.

Vol. IX, No. 4—December, 1914.

(Whole Series, Vol. XIX, No. 4.)

CONTENTS:

A STUDY OF THE HISTOLOGICAL VARIATIONS OF QUERCUS MUHLENBERGII, ... *John A. Elliott.*

PUBLISHED BY THE UNIVERSITY,

LAWRENCE, KAN.

Entered at the post office in Lawrence as second class matter.

5-2496

KANSAS STATE PRINTING OFFICE.
W. C. AUSTIN, State Printer.
TOPEKA. 1914.

THE KANSAS UNIVERSITY SCIENCE BULLETIN.

VOL. IX, No. 4.] DECEMBER, 1914. [WHOLE SERIES VOL. XIX, No. 4.

A Study of the Histological Variations of *Quercus muhlenbergii.*

BY JOHN A. ELLIOTT.

Plates XI to XV.

THE work, the result of which this paper will set forth, was done upon the suggestion and under the helpful direction of Prof. W. C. Stevens. Four native oaks growing in close proximity and similar in many characteristics showed some differences which made their classification rather doubtful. A study of the anatomy was undertaken as a more definite means of detecting constant similarities and variations.

One of the four filled the description of *Q. muhlenbergii* in Gray and Britton and Brown. The other three did not fit satisfactorily any description, so that in the study all three were designated by numbers, as 1, 2, 3 and 4. Specimens of each sent to the Gray Herbarium, Harvard University, were returned as all being *Q. muhlenbergii.* This made the designation by number as good as any as a basis for description.

MACROSCOPIC DIFFERENCES.

The leaves of No. 1 averaged smaller than those of the other trees and were more uniform in shape and size. The teeth of the serrate margin were sharply acuminate, averaging about ten on a side. The secondary veins were almost straight. The leaves of Nos. 2, 3 and 4 varied greatly in size and form, the teeth never being as sharply acuminate as in No. 1, and the leaves, especially of Nos. 2 and 3, being gen-

Received for publication May 11, 1914.

erally much larger. The secondary veins of No. 4 were nearly straight, those of No. 2 and 3 were curved. (Figs. 1 to 7.)

The acorns showed the most marked macroscopic difference between the four oaks. The acorns of No. 1 averaged 1.5 cm. in length and width; No. 2 averaged 2 cm. long by 1.5 cm. in width; No. 3 averaged 1.1 cm. in length by 1.2 cm. in width; No. 4 averaged 1.6 cm. in length by 1.2 cm. in width. (Figs. 8, 9, 10, and 11.) Excepting No. 4, the cups were much alike, being relatively deep and covered with thick bracts. No. 4 was shallow and covered with thin, tightly appressed bracts.

The seeds varied in size in about the same ratio as the acorns, except that No. 1 was relatively smaller on account of the greater thickness of the shell. (Figs. 8 to 11 and 12 to 15.)

The branches and twigs were much alike except that the epidermis of Nos. 1 and 3 split up much earlier than that of Nos. 2 and 4, making the twigs of the former rough while those of the latter were smooth.

The differences enumerated above formed the basis for the microscopic study of the anatomy.

PREPARATION OF MATERIAL.

Material was prepared for sectioning by fixing in Fleming's fixative and embedding in paraffin. Leaves were bleached in chloral hydrate and potassium hydrate. In the study of the wood, sections were made on the hand microtome from material preserved in formalin and softened in dilute glycerine. In tracing the middle bundle of the midrib, formalin-preserved material was used, the sections being made on the hand microtome.

BLEACHING.

There was a notable difference in the length of time it took to bleach the leaves in choloral hydrate. No. 1 bleached in 24 hours, No. 4 in 6 days, Nos. 2 and 3 in 7 days. In potassium hydrate No. 4 was bleached in 24 hours, the others in 48 hours. After bleaching, the leaves were stained 30 minutes in safranin and decolorized in 70 per cent alcohol, which aided greatly in differentiating the various tissues. Examination of the bleached leaves under the microscope immediately revealed the probable cause of the difference in time of bleaching. The walls of the lower epidermis of No. 1 were very thin, almost undistinguishable, while those of the others were relatively thick.

STOMATA.

The size and number of the stomata as revealed in the bleached leaves are features that enable one readily to distinguish between the four oaks. In all the leaves the stomata were more numerous near the midrib than near the edge of the leaf. By the usual method of averaging fields the count of No. 2 ran up to about 2000 per sq. mm. In order to get more definitely and accurately the number of stomata, 1 sq. mm. of leaf surface magnified 134 diameters was projected on paper and each stoma marked. (Figs. 23 to 26.) This showed the following numbers per sq. mm.: No. 1, 1074; No. 2, 1252; No. 3, 912; No. 4, 785. The stomata of No. 3 often occurred in rifts and clumps while those of the other oaks seemed more evenly distributed.

Average length of guard cells: No. 1, .0188 mm.; No. 2, .019 mm.; No. 3, .019 mm.; No. 4, .0189 mm. Average width: No. 1, .005 mm.; No. 2, .0059 mm.; No. 3, .006 mm.; No. 4, .0059 mm. Some of the largest stomata of No. 3 were four times as large as the smallest, a variability immediately noticeable. The others were more uniform in size.

The extreme minuteness of the guard cells and the fact that the stomal opening extends no more than a third of the length of the cells makes the mechanism of the stomata an interesting problem which I think is not fully explained by papers on the subject. The stomata are of the "gramineous" type, but looking down on a surface view of a stoma one sees deep depressions between the guard cells where these extend beyond the ends of the stomatal opening, and the heavy inner walls of the two guard cells facing each other here stand far enough apart to afford considerable room for movement when the cells become turgid (figs. 16 and 19, P and P′). The enlarged lumen at the ends greatly increases the surface of the inner heavy wall subject to the pressure of the cell sap, and these walls being free to move, as just shown, would undoubtedly move toward each other with the increase of turgidity. The points of contact of the two guard cells at the ends of the stomatal opening acting as fulcrums (fig. 16, O), the movement of the thickened walls must exert considerable leverage tending to open the stoma. (Figs. 16 and 18, r and r′.) This mechanism alone would seem to suffice to open the stoma, and it must be one of the most important factors, since a study

of the stomata shows that the smaller the stoma the relatively longer are the free-moving walls of the guard cells, so that the leverage is increased. Other parts of the mechanism of the gramineous type of stoma have been explained by other investigators.

In the very young leaf the stomata are not of the gramineous type, but are modified as the leaf matures. In the preserved material of the young leaf the stomata stand open, while they are never found open in the preserved material in the mature leaves. (Figs. 20 to 22.)

LOWER EPIDERMIS.

Aside from the stomata, the lower epidermis showed characteristic differences. As noted above, the cell walls of No. 1 were so thin as readily to distinguish it from the others, and the cells were also much smaller. No. 4 could be easily detected by its relatively large cells with wavy walls, while the walls of the other three were straight. The average area of the lower epidermal cells was as follows: No. 1, .000108 sq. mm.; No. 2, .000142 sq. mm.; No. 3, .000147 sq. mm.; No. 4, .00023 sq. mm. (Figs. 45 to 48, sec. D.)

EPIDERMAL HAIRS.

The number and character of the epidermal hairs are distinguishing features. No. 1 has 55 stellate hairs to the sq. mm., with 5 to 9 branches to a hair. They are small and very thin walled. No. 2 has 70 stellate hairs per sq. mm., with from 4 to 9 branches, and the hairs are generally long and thick walled. No. 3 has 215 stellate hairs per sq. mm., with 6 to 14 branches on each hair. These almost completely cover the surface of the leaf and make immediately distinguishable the smallest section of a leaf. The hairs of No. 3 are generally shorter and thicker walled than in No. 2. No. 4 has 80 hairs per sq. mm., with from 6 to 10 branches on each hair. Except in thickness of wall, they are like those of No. 2, but the walls are much heavier, having the central cavity a mere line. (Figs. 45 to 48, sec. E.)

UPPER EPIDERMIS.

The cells of the upper epidermis of the several oaks differed in size and thickness of walls. No. 1 had the thickest walls. The walls of No. 4 were nearly as heavy, while those of No. 2 were lightest. Comparative average area of upper epidermal

cells in sq. mm.: No. 1, .000542; No. 2, .000503; No. 3, .000604; No. 4, .000661. (Figs. 45 to 48, sec. A.)

MESOPHYLL.

In the bleached leaf the palisade cells of No. 2 were seen to be much more numerous and densely crowded together than in the other three oaks. The palisade cells of No. 1 were smaller than the others, but aside from these differences the mesophyll was of no value in classification. The spongy parenchyma was not discernable in the bleached leaf, but tangential sections from paraffin-imbedded material showed that the cells of No. 1 and No. 4 were generally smaller than those of Nos. 2 and 3. These cells, however, varied greatly in the same leaf.

In cross section the palisade cells of No. 2 were seen to vary greatly in length, while those of the other oaks were more uniform. (Figs. 27 to 30.)

VENATION.

Aside from the macroscopic differences already noted, the only constant differences in venation was in the distance apart of the vein endings. These varied in order: No. 2, 1, 3, 4, with No. 4 having the closest venation and No. 2 the most open venation. (Figs. 49 to 52.)

Oaks.	Epidermal hairs.	Stomata per sq. mm.	Lower epidermis.	Upper epidermis	Mesophyll.
No. 1.....	55 per. sq. mm. Very thin walled.	1074 generally uniform.	Average area of cells: .000108 sq. mm. Walls straight, very thin.	Average area of cells: .000542 sq. mm. Walls heavy.	
No. 2.....	70 per. sq. mm. Walls of medium thickness.	1252 generally uniform.	Average area of cells: .000142 sq. mm. Walls straight, of medium thickness.	Average area of cells: .000503 sq. mm. Walls quite thin.	Palisade cells closely compacted.
No. 3.....	215 per. sq. mm. Walls heavy.	912 variable in size and shape.	Average area of cells: .000147 sq. mm. Walls straight, of medium thickness.	Average area of cells: .000604 sq. mm. Walls of medium thickness.	
No. 4.....	80 per. sq. mm. Walls very heavy.	785 generally uniform.	Average area of cells: .00023 sq. mm. Walls wavy, of medium thickness.	Average area of cells: .000661 sq. mm. Walls heavy.	

This summary might serve as a key to classification, and with this idea in view all the oaks with leaves similar to the four types here studied found in the herbarium were examined.

by means of the bleached leaves. These were from various parts of the United States, and had been given various classifications: *Q. acuminata* (Tennessee), *Q. acuminata* (North Carolina), *Q. acuminata* (Lake Champlain), *Q. prinus* L. (Douglas, Kan.), *Q. prinus* L. (Florida), *Q. prinus discolor* (Illinois), *Q. castenea* Willd. (place of collecting not given).

A microscopic examination showed that those classified as *Q. acuminata* were identical with No. 1 of the four studied. Of the others, *Q. prinus* from Douglas, Kan., *Q. prinus discolor* from Illinois, and *Q. castena,* were identical with No. 3. *Q. prinus* L. from northern Florida was like No. 4 in every particular, with the exception of epidermal hairs, which were entirely lacking. In respect to the large lower epidermal cells with wavy walls, which was the most distinguishing characteristic of No. 4, it was identical. None was found like No. 2.

As a further check on this method of classification, sections of leaves from nine different specimens of *Q. prinoides* found in the herbarium were taken and bleached as before. This oak was selected because of its resemblance to those studied. One of the specimens was from Douglas, Kan., one from Illinois, and the other seven were collected near Troy, Kan., in September, 1913. By the methods used in the study of the four oak under investigation it was found that *prinoides* has 1023 stomata per sq. mm. The epidermal hairs are like those of No. 2, except that they were uniformly much shorter. The palisade cells were closely compacted, as in No. 2, and the cells of the epidermis similar to those of No. 2. The smaller branches of the veins were much more prominent than those of any of the four oaks being studied. These characteristics held good for every specimen of the nine taken, they being so exactly alike that there was no variation that would distinguish one from the others.

This seemed enough evidence to conclude that the wide and constant differences found in the four oaks under consideration were constitutional and not modificational variations.

PETIOLE AND MIDRIB.

Figures 34 to 37 show cross sections of the petioles of the leaves at the base of the leaf blade. They show some differences in the shapes of the petioles and of the various tissues, more especially in the shape of the middle bundle. In Nos. 3 and 4 the bast, phloem, and xylem regions make continuous

concentric bands, while in Nos. 1 and 2 the xylem and phloem bands are broken, so that the vascular system is in three bundles. The middle bundles vary greatly in shape, but there is considerable variation in this respect in different petioles from the same individual oak.

Figures 27 to 30 show cross sections of the midrib of the leaves taken at two-thirds up from the base of the blade and a little above the insertion of a vein, which accounts for the bast ring being broken. The sections show that the middle bundle of Nos. 1, 3 and 4 have disappeared, while that of No. 2 still persists.

Cross sections were made on the hand microtome, from material preserved in formalin, to determine the manner and place of the ending of the middle bundle. This method was used as being better than embedded material in keeping the exact position of each section taken. My fellow student, F. W. Mulsow, found that a mixture of safranin and hæmatoxylin makes a very good double strain and greatly shortens the process for temporary mounts. A section can be kept on the point of a needle, dipped in the double strain for a few minutes, washed in seventy per cent alcohol, and mounted in glycerine, with very satisfactory results.

The middle bundle of No. 2 ended a little above two-thirds the length of the leaf, that of No. 4 a little below two-thirds the length, and that of Nos. 1 and 3 a little above one-half the length of the leaf. The size of the leaf and midrib made no difference in the relative distance the middle bundle ascended the midrib. At the point of ending of the middle bundle in a large leaf the midrib might be larger than the midrib at the base of the blade in a smaller leaf.

The branching of the midrib into the lateral veins has an indirect effect upon the middle bundle, as the bundles break up into sections whenever a branch is given off, and in the readjustment a portion of the middle bundle is attached to the upper bundle. The middle bundle does not branch directly into the secondary veins. The termination of the middle bundle in No. 4 is distinctly different from that in the other three oaks, as the middle bundle in No. 4 always disappears by joining to the side of the upper bundle, while the juncture in the other three is in one manner or another always made at the middle of the upper bundle. The manner of joining

is essentially the same in Nos. 1, 2, and 3, although in this respect there are variations in leaves of the same tree. (Figs. 31 to 33.)

ACORNS.

The cells of the acorns were approximately the same size, but there was considerable difference in the sizes of the starch grains. The average diameters of the starch grains were as follows: No. 1, .00784; No. 2, .006; No. 3, .00545; No. 4, .00645 millimeters. The cells of the acorns were crowded with starch grains in all cases. (Figs. 38 to 41.)

Iodine, nitric acid and ammonia, and Millon's reagent were used to test for proteins. All three reagents showed more protein in seeds from No. 2 than in the other three, which were about equal in this respect. Ferric chloride indicated about equal amounts of tannin in all, but this was not as abundant in these acorns as in those of most oaks.

Macerations of the shell and cup of the acorns showed a great variety of stone cells and tracheids, but nothing that would distinguish one from the others. (Figs. 42 to 44.)

WOOD AND BARK OF THE CURRENT YEAR.

A cross section of the current year's growth of the stem shows an even gradation in diameter from No. 1 to No. 4. This is mostly due to the differences in thickness of the regions outside of the cambium ring. The growth of the xylem is variable in the same specimen, but the thickness of bark is constant. In No. 1 and No. 3 the cork-cambium produces enough cork in the current year's growth to break up the epidermis, while Nos. 2 and 4 have a smooth epidermis, that of No. 4 persisting for at least three years. The greatest differences were noticeable in the widths of the parenchyma tissues of cortex and pericycle. The bast ring varies in average width as shown in the following measurements: No. 1, .087 mm.; No. 2, .06 mm.; No. 3, .0706 mm.; No. 4, .0435 mm. The average distances between cambium ring and bast ring are: No. 1, .15 mm.; No. 2, .12 mm.; No. 3, .07 mm.; No. 4, .07 mm. The average widths of the parenchyma of cortex are: No. 1, .22 mm.; No. 2, .18 mm.; No. 3, .11 mm.; No. 4, .10 mm. In amount of pith Nos. 1 and 4 were about equal and they had much less than Nos. 2 and 3. The pith cells were about the same size in all. All these variations were immediately noticeable under the microscope. (Figs. 53 to 56.)

OLDER WOOD AND BARK.

In the study of the more mature stem sections were taken from the oldest material at hand. The cross sections showed that the differences in thickness of bark persisted, but that the relative increase in thickness of bark due to the cambium ring was about the same. The original bast ring of No. 2 remained entire for at least seven years. That of No. 4 was unbroken at three years. (Older material not available.) The bast ring of No. 3 was broken up in the four-year-old stem. That of No. 1 was never entire. (Figs. 61 to 64.)

No. 1 showed the slowest growth of xylem and No. 3 the most rapid, from trees very nearly all the same size. Cross sections of the wood showed nothing that would distinguish any one of the oaks except that the medullary rays of Nos. 1 and 2 were wider than those of Nos. 3 and 4. (Figs. 65 to 68.) In tangential section this showed much more clearly and was an identifying characteristic. The sections were taken from growth of the same year. (Figs. 57 to 60.) In the same area (1.04 sq. mm.), the number of medullary rays was as follows: No. 1, 129; No. 2, 63; No. 3, 134; No. 4, 144. The difference between Nos. 1 and 4 was least pronounced. No. 3 could be immediately identified by its very narrow rays, and No. 2 by its large, broad ones.

Macerations of the wood and bark revealed no distinguishing characteristics.

SUMMARY.

The four oaks under consideration, classified as all being *Q. muhlenbergii,* show some difference in general appearance of leaves and acorns. The histology of the leaf reveals constant identifying differences in epidermal hairs, number of stomata, and size of epidermal cells, any one feature of which will distinguish any one individual from all of the others. In addition to these features which are different in each oak, No. 4 is distinguished by wavy-walled cells of the lower epidermis; No. 1 by thin-walled epidermis, and No. 2 by closely packed palisade cells. The manner of ending of the middle vascular bundle of the midrib differentiates No. 4, and the place of ending differentiates No. 2.

There is a difference in the sizes of the starch grains in the seeds. No. 2 is characterized by the presence of the most protein in the seeds.

The four oaks may be distinguished by the thickness of the bark in the current year's growth, and by the tissues of the bark.

There is a distinguishing difference in the number and size of the medullary rays in the wood.

CONCLUSIONS.

No. 1 is the species described as *Q. muhlenbergii* and may be identified macroscopically by its narrow leaves with sharply acuminate, serrate teeth. The shell of the nut is twice as thick in No. 1 as in Nos. 2, 3, and 4. Nos. 2 and 3 are possible hybrid *muhlenbergiis*. They may be distinguished from No. 1 by their broader and more irregular leaves. No. 2 has large acorns, and has a smooth bark for several years, while the epidermis is broken up on Nos. 1 and 3 in the current year's growth. The acorns of No. 3 are very small and the nut is almost overgrown by the cup. A hand lens will reveal the unusual number of epidermal hairs on the lower epidermis of No. 3, which might well give it the variety name of "hirsuta."

No. 4 is enough different from the others to be classed as a distinct species. It may be identified macroscopically by its slightly paler leaves, smooth-barked twigs and a marked difference in the acorn cup, which is shallow, thin, and covered with thin tightly appressed bracts, while those of Nos. 1, 2, and 3 are generally deeper, thicker and covered with thick bracts which make the cups irregular and rough. Either No. 4 or No. 2 is probably *Quercus alexanderi* of Britton and I would suggest that name be retained for No. 4.

BIBLIOGRAPHY.

COPELAND, Mechanism of Stomata, Ann. of Bot. XVI, 1902, p. 327.

SCHWENDENER, Bau u. Mechanism. d. Spaltoffn., Berliner Akad., 1881, p. 833.